CCII. DISTRIBUTION OF PHOSPHATASE ACTIVITY AND ANALYSIS OF GROWTH IN CANADA WONDER BEAN

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(Received 9 June 1937)

IN a previous publication reporting on the distribution of phosphatase in the plant of Canada Wonder bean Ignatieff & Wasteneys [1936] suggested that phosphatase may be concerned with catabolic as well as anabolic processes of carbohydrate metabolism of the plant. The growth of the plant depends to a very great extent on the carbohydrate metabolism because the chief constituents of the plant dry matter are products of this metabolism. Therefore a growth analysis was made on the same plants which were used for the study of the distribution of phosphatase.

EXPERIMENTAL

The method for the determination of phosphatase activity as well as its distribution in the Canada Wonder bean grown in culture solution has already been reported in the above-mentioned paper, and therefore only the details of the growth analysis of the plants will be discussed here. For the growth analysis the formulae suggested by Briggs *et al.* [1920] were used. In calculating the results it was assumed that the increase in dry matter in leaf area was on the linear and not on the exponential basis. The linear basis has been adopted as the simpler one and as being sufficiently accurate for the purpose. According to Briggs *et al.* nothing is gained by the use of the more complicated formulae.

Sampling was performed at approximately 1 week intervals and nine to ten plants were used at each sampling. Each plant was divided into leaves, stem and root. The weight of each of these parts was recorded separately for the individual plant. By this means it was possible to submit the results obtained for each plant to statistical analysis and ascertain the probable error of the mean. As seen from Table I the probable error of the mean is very small.

The area of leaves was calculated from their blue prints. The leaves of nine to ten plants taken for sampling were then collected into three groups: the primary leaves, the older compound leaves and the newly formed compound leaves. These three types of leaves were separately ground into a paste which was spread on clock glasses and dried at room temperature in a rapid current of air. The stems and the roots were similarly treated.

The dried material was then weighed and samples were taken for the determination of phosphatase activity. The moisture content of the dried material was determined, and from these determinations it was possible to obtain the actual dry matter of the different parts of the plants.

The area of the leaves in cm. and the fresh and the dry weight in g. of the different parts of a single plant are given in Table I, where are also presented the results of the growth analysis and the phosphatase activity of different parts of the plant.

36	Primary leaves Older compound leaves Newly forming compound leaves Stem Root	4.04 4.85 5.01 1.98 1.04	9.32 $\pm$ 0.18 4.79 $\pm$ 0.08 2.53 $\pm$ 0.05	1.041 0.516 0.179	483				
42	Primary leaves Older compound leaves Newly forming compound leaves Stem Root	2.91 3.27 4.12 1.59 0.68	11.76 $\pm$ 0.30 5.76 $\pm$ 0.11 3.30 $\pm$ 0.08	1.236 0.642 0.174	581	21.1	283	0.68	19.2
49	Primary leaves Older compound leaves Newly forming compound leaves Stem Root	3.02 3.16 4.14 1.79 0.94	12.36 $\pm$ 0.45 6.06 $\pm$ 0.14 3.26 $\pm$ 0.08	1.56 1.05 0.20	662	13.0	255	1.22	31.1
58	Primary leaves Older compound leaves Newly forming compound leaves Stem Root	2.01 2.49 3.00 1.37 0.99	15.64 $\pm$ 0.73 7.82 $\pm$ 0.38 3.88 $\pm$ 0.17	2.07 1.35 0.30	722	6.9	217	1.03	22.3
66	Primary leaves Older compound leaves Newly forming compound leaves Stem Root	2.37 2.36 2.91 1.65 Trace	14.89 $\pm$ 0.81 7.55 $\pm$ 0.38 4.02 $\pm$ 0.31	2.07 1.62 0.29	699	0.0	185	0.32	6.0
78	Pods Older pods Primary leaves Older compound leaves Newly forming compound leaves Stem Root	1.67 1.68 2.79 2.19 2.56 1.00 Trace	16.50 $\pm$ 0.98 9.68 $\pm$ 0.36 6.31 $\pm$ 0.29	2.25 2.17 0.59	781	5.7 5.4	170 167	0.71 0.67	12.0 10.7

In Fig. 1 are graphically presented the values for the relative growth rate, for the unit leaf rate and for the phosphatase activity of the older compound leaves.

Fig. 1. Phosphatase activity of the older compound leaves, the relative growth rate and unit leaf rate of the bean plant grown in water culture solution. Ordinates: 1, mg. inorganic P released from sodium β -glycerophosphate solution by phosphatase in 1 g. of dry matter per minute. 2, Unit leaf rate in mg. of dry matter per 1 sq. cm. of leaf area. 3, Relative growth rate expressed as percentage increase in dry weight. Abscissa: days after planting. — Phosphatase activity. ---- Unit leaf rate. ++++ Relative growth rate.

As the area of the older compound leaves is much greater than that of the other types of leaves during the greater part of the life cycle of the plant, the older compound leaves must exert a much greater influence on the metabolism of the plant than do the primary leaves or the newly forming compound leaves. For this reason the results for the phosphatase activity in older compound leaves were selected for the graph. Because the values for unit leaf rate indicate the efficiency of the leaves to form dry matter and the values for the relative growth rate give a direct indication of the rate of increase in dry matter of the plant, they have been included in the graph.

DISCUSSION

From the results presented in Table I and Fig. 1 it is seen that the phosphatase activity in the leaves, after reaching an optimum 35 days after planting, gradually declines. This decline in activity may be due to two causes. The metabolism of the leaf cells may become less active with increase in age of the plant, or it may be that the decline of phosphatase activity in the leaves is more apparent than real and is due to the incorporation of dead fibre amongst the leaf cells. The phosphatase activity was determined on the basis of 1 g. of dry matter. If there is a gradual increase of inert material in plant tissue, there will be a corresponding decrease of living cell matter per unit of dry matter. That the decrease of phosphatase activity is probably due to the decline in the metabolic rate of the living plant cells is shown by the trend in the enzymic activity of the newly forming compound leaves. In these leaves the enzymic activity is a little higher than in the older compound leaves, but with the increase in age of the plant the phosphatase is not as active in newly forming leaves as it was when the plant was young.

In newly forming leaves the amount of inert fibrous material is negligible, and therefore it is reasonable to suppose that there is actually less phosphatase activity in the cells of the leaf.

From the curves in Fig. 1 it will be noticed that the values for the unit leaf rate and the relative growth rate follow the same trend as those for the phosphatase activity in the older compound leaves. The maximum for these values is reached at the same time as the phosphatase shows its maximum activity in the leaves, and these maximum values are followed by a rapid decrease in phosphatase activity and in the production of dry matter.

As the plant ages the enzymic activity decreases, but the decrease is not as rapid as it was when the plant was 5 or 6 weeks old. The same can be said for the values of unit leaf rate and relative growth rate, although the curves for these values are not as regular as the curve for phosphatase activity.

The similarity of the curves for the unit leaf rate, for the relative growth rate, and for the distribution of phosphatase activity in the older compound leaves, suggests an interrelation between growth of the plant and phosphatase activity in the leaves. It has already been stated that the chief constituents of the plant dry matter are products of carbohydrate metabolism, and therefore the growth of the plant must be directly proportional to that metabolism. The apparent interrelation of phosphatase activity in the leaves and growth of the plant can be taken as further evidence that this enzyme is concerned with carbohydrate metabolism.

The growth analysis, in addition to lending support to this hypothesis, is of further value. In a study of the distribution of an enzyme, which entails the whole life cycle of the plant, it is difficult to adopt some method for the correlation of the enzyme's activity with definite stages of development of the plant. One method which is frequently used is to correlate the metabolic activity of the plant with intervals of time during which growth takes place. However, this method is not very accurate because it is well known that during any given period of time, under varying conditions, the growth and the development of the plant vary considerably.

By the use of the methods advocated by Briggs *et al.* a direct correlation can be made between the distribution of phosphatase activity and the growth of the plant.

SUMMARY

1. Studies of the distribution of phosphatase activity and analysis of growth in Canada Wonder bean grown in culture solution throughout its life cycle were made.

2. The unit leaf rate and relative growth rate show a very close correlation with the phosphatase activity of the leaves.

3. The close correlation between the increase of dry matter in the plant and the phosphatase activity of the leaves has been suggested as further evidence that phosphatase plays a role in carbohydrate metabolism.

The author wishes to express his gratitude to his friend Dr L. B. Pett of the Biochemistry Department, University of Alberta, for his advice and assistance with the statistical analysis.

REFERENCES

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